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October 1917. BACTERIOLOGICAL SERIES. VOL. I, NO. 7.

MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA

A BACTERIAL DISEASE OF WHEAT IN THE PUNJAB

BY

C. M. HUTCHINSON, B.A

Imperial Agricultural Bacteriologist

AGRICULTURAL RESEARCH INSTITUTE, PUSA

PRINTED AND PUBLISHED FOR

THE IMPERIAL DEPARTMENT OF AGRICULTURE IN INDIA

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A BACTERIAL DISEASE OF WHEAT IN THE PUNJAB.

BY

C. M. HUTCHINSON, B.A.,

Imperial Agricultural Bacteriologist.

[Received for publication on 14th May, 1917.]

I. Introduction.

Tannan or Tandu is the vernacular name locally used for a disease of wheat which has been known for many years in the Punjab. The writer's attention was first directed to it by the Imperial Mycologist in 1911 and examination of specimens confirmed the latter's previous diagnosis of its bacterial origin. Owing to the distance separating Pusa from the Punjab, further investigation was delayed until the writer visited Lyallpur in March 1913 and obtained fresh specimens, but inoculation tests have not been fully carried out owing to the fact that the disease does not appear in the field until the crop is nearing maturity, which in the Punjab is at too late a season of the year to make it possible to bring fresh material from thence to Pusa and there carry out field experiments in the same year, the wheat-growing season in Bihar being several weeks earlier than in the former locality. It has been thought, however, that publication of a description of the disease and of the characters of the causative organism so far as they have been ascertained, would be of interest to plant pathologists, more especially in view of the recently published description of a similar bacterial disease of wheat grass, *Agropyron Smithii*, by O'Gara in North America—(*Phytopathology*, Vol. VI, No. 4).

II. The Disease: Its Description and General Characteristics.

The principal characteristics of this disease in the Punjab are very similar to those described by Rathay and O'Gara in the case of orchard grass, *Dactylis glomerata*, and wheat grass respectively. The inflorescence and parts of the

stem are covered with a bright primrose yellow slime or gum, forming adherent sticky layers between the glumes and between the stem and sheath. This slime is composed of masses of bacteria and the outer exposed portions become dried up, hard, and flaky, and at the same time take on a deeper yellow tone. A frequent characteristic, due to the interference of the sticky bacterial masses with the growth and expansion of the plant, is the distortion of the stem immediately below the head, two typical forms of which are shown in Plate I.

Although the disease has appeared in the same district (Montgomery) of the Punjab every year since 1908, with one exception in 1915, and is reported as having been known there for many years previously, it does not appear at present to be of serious importance, as it affects only a very small percentage of plants and those only in badly cultivated or badly drained and consequently infertile soils. It will, however, be necessary to keep the incidence of this disease under continual observation in order to ensure against any acquisition of greater parasitic activity and with it of extended distribution of the causative organism; the use of improved varieties of plant might easily afford greater facilities for attack as compared with those presented by the present acclimatized strains of wheat. It may be said, however, that any such spread of the disease is much less likely to occur in the comparatively arid districts of the Punjab where wheat-growing depends upon irrigation, than in other countries where natural soil moisture is found throughout the year, such moisture not only lowering the strain upon the vitality of the organism by reducing the length of the resting period induced by, and necessary to survive desiccation, but also ensuring the absence of abnormally high soil temperatures.

A further cause for freedom from anxiety on this account exists in the very high soil temperatures reached in this part of India and persisting for considerable periods. As the writer pointed out in connection with the Rangpur Tobacco Wilt (*Memoirs of the Dept. of Agric. in India, Bact. Series*, Vol. I, No. 2), the high soil temperature in Eastern Bengal at the time of ploughing after cutting the tobacco reduces the number of bacteria parasitic on this plant and surviving in the soil, to a minimum, owing to the comparatively low thermal death point of *B. solanacearum*. Similarly the organism isolated from the diseased wheat plants was also found to have a low thermal death point (about 50° C.) resembling *A. agropyri* in this particular (*loc. cit.*), and this fact, together with the difficulty of keeping it alive for any length of time in culture at any temperature above 20° C., makes it very improbable that this organism will become of economic importance, at any rate in that part of India

Bacterial disease of wheat in the Punjab caused by *Ps. Tritici.*

in which alone it has so far been found. This conclusion is strengthened by the fact that the disease occurs in patches, and, as above described, is practically confined to badly drained areas where in all probability not only is cultivation and drying out of the soil imperfectly carried out, but where the higher humidity of the air during the growing season of the crop would tend to ensure the deposition of dew in sufficient quantity to promote the growth of the organism on the plant.

As described later in this paper in connection with infection experiments, it was found impossible to obtain growth of the organism on wheat plants at Pusa except under a belljar, the use of the latter ensuring a sufficiency of moisture in the air and the formation of dew on the plant.

Resistance to desiccation varied greatly; it was found easy in some cases to obtain growth on culture media from dried specimens of more than seven months age, whereas cultures on agar were with difficulty kept alive by repeated transfers and eventually failed to survive through the dry hot season of the year even although kept in a cool incubator at 20° C. It appears probable therefore that this disease is kept in check in the only part of India where it is known at present, by natural causes such as the dryness of the air and high temperature of the soil, and as these are invariable except in the case of a decided seasonal change of climate, the only chance of serious damage to the wheat crop from this cause in the Punjab would lie either in the injudicious use of irrigation water or the introduction of a variety of plant much more susceptible to the attacks of this parasite. A third alternative is the possible acquisition of higher parasitic power or resistance to adverse climatic conditions on the part of the bacterium; fortunately experience does not lead us to anticipate any such variation in the characters of a bacterial parasite under natural conditions as is known to occur in the case of insect pests, so that although it is possible to raise the virulence and general parasitism of bacteria by artificial cultural methods, the necessary conditions for this result are not likely to occur in nature or even under the semi-artificial methods of the cultivation of wheat under irrigation.

The dissemination of the organism may be either through individuals persisting in the soil or from those carried on the grain or chaff of the plant. In view of the continued vitality mentioned above as characteristic of the latter source, it is probable that this might be a source of danger to other districts, where conditions for growth and spread of the parasite might be better than they are in the Punjab. This possibility should be kept in mind in using seed wheat from infected districts.

In view of the importance of the wheat crop in India, the further investigation of the conditions under which infection takes place and the relations of host and parasite should be carried out most thoroughly. It may be pointed out that infected grain might serve as an originator of this disease in countries having temperate climates where no natural checks, such as those above described, exist. This point requires careful investigation, and if it were shown that this disease was capable of attacking the wheat plant in temperate climates, very drastic regulation by Government of the export of wheat from infected areas would be necessary.

It has been suggested to the writer by Mr. Milne, Economic Botanist to the Government of the Punjab, that the incidence of this disease depends upon mechanical injury to the plant caused by eelworms, and his study of the subject leads him to conclude that without such intervention the disease itself does not occur. This is an interesting suggestion and worthy of due consideration in view of the well-known intermediary action of such organisms as eelworms and beetles in connection with other bacillary diseases of plants. It will be seen, however, from study of the sections illustrated that independent penetration of certain tissues by the bacterium probably occurs (Pl. III, fig. 1); Mr. Milne suggests that bacterial growth on the plant only takes place in the exudate resulting from penetration by the eelworm and that the latter must therefore be considered the causative organism of the diseased condition; this view of the case would necessitate the somewhat wide assumption that the specific bacteria invariably found in the gummy masses characteristic of the disease and capable of producing them in artificial culture, are omnipresent in the affected areas, but only multiply in the characteristic manner when associated with eelworm attack. In any event the characteristic distortion of the plant and failure to make normal growth appear to be mainly due to the formation of the gummy deposits resulting from bacterial activity, from which it may be concluded that the disease is due to bacterial action although nematodes may play an important part either in conveying or in accentuating it.

III. Description of the Bacterial Parasite.

Yellow organism isolated from wheat ears suffering from Tandu disease. April, 1913.

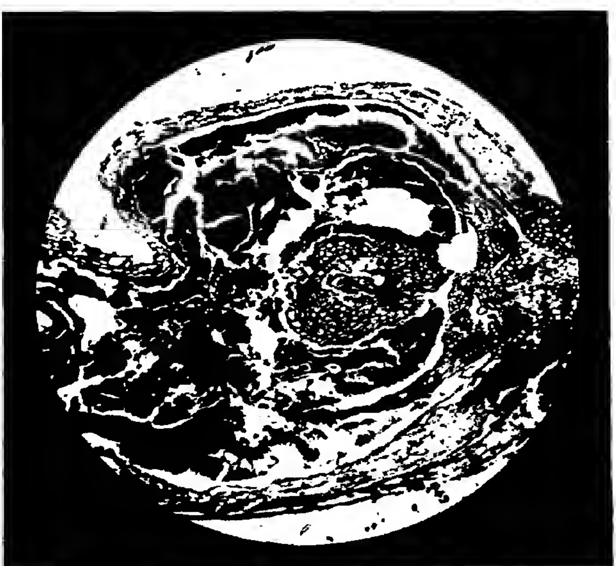
Motile : 1 Polar flagellum.

Size $\cdot 8\mu \times 2\cdot 4$ to $3\cdot 2\mu$.

Ordinary Agar (+5 Fuller) Colonies ..	Bright yellow colour after 24 hours, turning slightly orange after 2 or 3 days, convex, round with entire edges granular, shining, moist, opaque in the centre, opalescent at the edges.

INTRACELLULAR BACTERIAL MASSES.
Zeiss 4 mm. apo.

INTRACELLULAR BACTERIAL MASSES
Zeiss 4 mm. apo.



SECTION OF WHEAT SPIKELET SHOWING DARK BACTERIAL MASSES.
Zeiss 16 mm. apo.

Agar streak	.. Scanty growth after 24 hours increases after 2 or 3 days. Yellow, moist, raised, shining, precipitate in the condensation water. Agar turned brown after 5 or 6 days.
Agar stab	.. Growth on the surface yellow, raised, shining, moist, becoming dry after some 6 or 7 days. Agar turned brown.
Gelatine stab (+10 Fuller)	.. Slight growth along the track of the needle: gelatine not liquefied even after 30 days. Surface growth moist, yellow, button-shaped.
Bouillon + 5 Fuller ..	.. Cloudy yellow precipitate at the bottom; thin pellicular ring at the side of the tube; alkaline reaction after 10 days.
Peptone water + 5 Fuller	.. Cloudy, no pellicle, except a thin ring at the side of the tube. Yellow precipitate.
Glucose Peptone water + 5 Fuller	.. Cloudy, yellow precipitate, no gas, gives a decided acid reaction at the end of ten days.
Lactose Peptone water ..	.. Cloudy, no gas, thin ring at the side of the tube. Yellow precipitate; slight acid reaction.
Litmus Milk	.. Slight yellow precipitate at the bottom after 24 hours. Cream layer at the top coloured yellow after 48 hours. Litmus reduced after 3 or 4 days. Litmus not blued even after 30 days.
Potato	.. Slight yellow growth not spreading beyond the line of inoculation; growth is only slightly increased with age. Alkaline reaction to litmus: No H_2S evolved.
Potato + Diastase ..	.. Growth copious, yellow, shining, moist, spreading: No H_2S evolved, Potato water cloudy.
Potato Starch Agar ..	.. Poor growth, yellow, moist, shining, slight precipitate in condensation water.
Potato Starch Agar + Diastase	.. Abundant yellow growth with precipitate in the condensation water.
Nitrate Broth.. ..	.. Nitrate reduced to nitrites and ammonia.
Coconut flesh ..	.. Abundant yellow growth; reaction was found to be alkaline to litmus. No difference between growth on autoclaved and unsterilized coconut.
Carrot (unsterilized) ..	.. Good growth showing a dark brown discoloration after 3 or 4 days after which rotting began.
Carrot (sterilized) ..	.. Good growth; yellow; no rotting; reaction acid to litmus.
Onion	.. Vigorous growth, gives an alkaline reaction.

Yellow pigment—intracellular; soluble in alcohol, insoluble in water or ether.

The first agar cultures made and incubated at 30° C. were found to be dead after about 10 days; after a few generations this period shortened to 3 or 4 days. At 20° C. the earlier cultures could be kept for 20 to 30 days but in later generations hardly over 8 to 10 days.

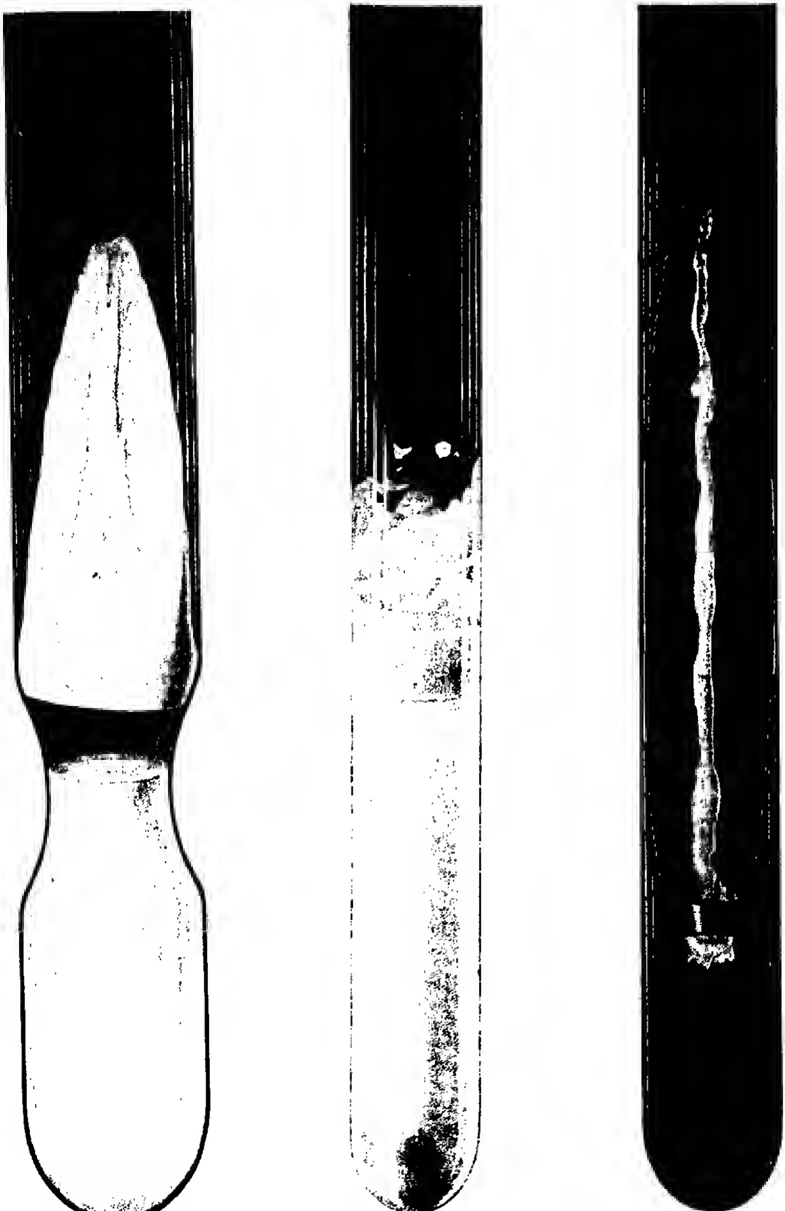
Thermal death point very low—about 50° C.

Suggested name—*Pseudomonas Tritici* sp. nov.

IV. Inoculation Experiments.

Growth was only obtained by inoculation on wheat plants, when the latter were kept in an abnormally moist atmosphere which could only be secured, during the season of the year in Bihar at which wheat can be grown, namely, the winter months from November to March, by covering the plants with a belljar. Under these conditions bacterial growth with formation of yellow slime took place, but the rapid growth of the wheat not only made it impossible to keep the plants under the belljars sufficiently long to observe the full effect of the parasite, but appeared to prevent the characteristic distortion accompanying the diseased condition observed in the field in the Punjab.

No successful inoculation of other graminaceous plants was secured, but a considerable amount of growth in living onion bulbs was obtained. This plant was selected on account of the numerous points of similarity in morphological, cultural and physiological characters, between the wheat bacillus and *Ps. Hyacinthi* Wakker, the onion being the nearest approach to the hyacinth available in the plains of East India. It will be noticed that the parasitism of the wheat bacillus is considerably reduced by its weak diastatic power; on the other hand, although also incapable of liquefying gelatine, it shares with other parasitic bacteria the power of breaking down proteins with the production of an alkaline reaction eminently favourable not only to its own growth but to that of other purely saprophytic bacteria, whose combined physiological activities exaggerate the diseased condition of the host. This multiple infection is a leading feature of plant disease in India, where climatic conditions generally favour its occurrence, and where the separation of the original invading parasite from its numerous saprophytic followers generally involves a long and tedious series of operations.



b.

Pure cultures of *Ps. Tritici* on (a) Potato (b) Litmus milk. (c) Agar.

V. Remedial Measures.

In view of the relatively small percentage of the crop affected by this disease it is unnecessary to do more than point out the value of drainage and thorough cultivation in dealing with this as with many other plant diseases of bacterial origin. In this case drainage is indicated not only on account of its effect upon the tilth and general condition of the soil but because of the influence of desiccation upon the vitality of the parasitic organism. For a similar reason thorough cultivation will not only adversely affect the bacterium by drying out the soil but will tend to destroy it altogether by raising the temperature of portions of the latter above the thermal death point of the parasite.

In conclusion it may be repeated that although there is reason for thinking that this bacterial disease of wheat is never likely to have serious effects in the districts where it is now found, yet careful watch should be kept lest its importation into other wheat-growing areas where climatic conditions are more favourable to its growth and persistence, should result in more decided damage.

Pusa:
May, 1917.

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November 1920. BACTERIOLOGICAL SERIES. VOL. I, No. 8.

MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA

PEBRINE IN INDIA

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PRINTED AND PUBLISHED FOR

THE IMPERIAL DEPARTMENT OF AGRICULTURE IN INDIA

BY

THACKER, SPINK & CO., CALCUTTA

W. THACKER & CO., 2, CREED LANE, LONDON